HISTOLOGY AND CYTOCHEMISTRY OF HUMAN SKIN

IV. The Eccrine Sweat Glands*

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Since the descriptive accounts of the morphology of eccrine sweat glands are vague and contradictory (1, 2), there is need for a clear statement of their cytology. In this paper we present a study of the fine structures and cytoplasmic inclusions of eccrine secretory cells. Special attention is paid to the cells of the excretory ducts, which abound in glycogen, basophilic granules and mitochondria; and it is suggested, in agreement with Lobitz and Mason (3) that the ducts may have an active function in addition to conveying sweat to the surface.

MATERIAL AND METHODS

Approximately 50 biopsy specimens from normal women, ranging from twenty-six to thirty-nine years of age, were removed without anesthesia from the skin of the axilla with a high-speed rotary biopsy punch (4). Nearly 50 surgical specimens from various regions of the body of male and female subjects were also used. All specimens were fixed at once after excision. For the study of mitochondria, tissues were fixed in Helly's or Regaud's fluid, and then postchromed for seven days in 3% potassium dichromate. Sections cut at 3 μ were stained with Mallory's phosphotungstic acid hematoxylin, with Regaud's hematoxylin or with Altmann's aniline-acid fuchsin-methyl green. Glycogen was revealed with the method of McManus (5) in 5 μ sections of Helly-fixed tissues. To obtain a clear localization of glycogen and to avoid indiscriminate coloration of the surrounding tissues, sections were stained for one hour in 2 ml of Schiff reagent diluted with 48 ml of the sulfurous acid rinse. Control sections were treated with saliva for 15 minutes before the application of the test.

For the study of basophilia and metachromasia, 5 μ sections of Helly-fixed tissues were stained in 0.05% toluidin blue buffered from pH 4.0 to pH 8.0 (for technique, see Montagna et al., 6). Cytoplasmic basophilia which is revealed with toluidin blue buffered to pH 5.0 is abolished in sections previously incubated for 3 hours at 60° C in 0.01% ribonuclease, buffered with McIlvaine's buffer to pH 6.5. When stained in toluidin blue buffered from pH 6.0 to 8.0 there is little diminution of cytoplasmic basophilia. We assume then, that the cytoplasmic basophilia obtained at pH 5.0 represents ribonucleic acid. All of the descriptions of basophilia in this paper are based on sections stained at pH 5.0. Since Montagna et al. (6) have already described the metachromatic substances present in human skin, only brief reference will be made to them in this paper. Sample sections of all the material studied were also stained with Giemsa stain. Paraffin sections of Helly-fixed tissues were routinely colored with Sudan black for the demonstration of non-extractable lipids.

Lipids were studied in tissues fixed in formol-calcium and subsequently postchromed in

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Baker's (7) dichromate calcium. Tissues were embedded in gelatin and sectioned at 5 to 10 μ. Sample sections were treated with each of the following routines: (a) colored with Sudan black; (b) extracted 10 minutes in acetone, one hour in chloroform, 10 minutes in acetone, brought back to 70% alcohol, and then colored with Sudan black; (c) extracted for 18 hours in pyridine at 60°C and then colored with Sudan black; (d) stained with Baker's (7) acid hematin test for the demonstration of phospholipids. Untreated frozen sections and comparable sections extracted in chloroform (see step b above), of material fixed only in formol-calcium were observed under near-ultraviolet light for autofluorescence and under polarized light for anisotropic lipids. Eccrine glands contain no birefringent lipids and no further reference will be made to this.

OBSERVATIONS

The secretory tubules of eccrine sweat glands are lined by one layer of truncated pyramidal cells. The cells are of different sizes and often appear to be arranged in two rows: large cells with basal nuclei and small cells, the nuclei of which are near the distal end of the cytoplasm (fig. 3). The disposition of these cells has lead Ito and Iwashige (2) to distinguish the larger ones as "basal" cells, which, according to them, rest upon the basement membrane and do not reach the lumen, and "superficial" cells which are found upon, or between the basal cells and which line the lumen. Actually all of the cells, "basal" and "superficial", rest upon the basement membrane and reach the lumen (fig. 2). The cytoplasm of the smaller cells is strongly basophilic, but that of the larger cells s not, and for this reason we prefer to call them dark and clear cells (figures 1 and 2). A loose mesh of myoid cells, described in detail by Bunting et al. (8), is interposed between the secretory cells and the thick hyalin basement membrane. There is a gradual transition between the cells in the secretory coil and the duct. The transitional region is composed of two layers of cells, basal cuboidal ones, and surface cells, the apices of which show a faintly yellow, hyalin cuticle. The ducts maintain two layers of cells as they ascend to the surface, but the cuticle becomes thicker and more distinct. Near the pore, the cuticle is gradually lost and the epithelium of the duct becomes pluristratified. In some regions of the body such as the scalp, the ductual "pore" appears to have its own lining, in agreement with Holyoke and Lobitz (9), in others, such as the axilla, the epi-

PLATE 1

Explanation of Figures

All figures in this plate are of tissues fixed in Helly's fluid and stained with 0.05% toluidin blue buffered to pH 5.0.

Fig. 1. Secretory eccrine tubule to show the distribution of dark and clear cells. From the axilla of a woman 26 years old. Ca. 675×.

Fig. 2. Detail of dark cells to show that they rest on the basement membrane. The clear cell at the upper left reaches the lumen; the three dark cells in the center have a cuticular border. From the axilla of a woman 38 years old. Ca. 2000X.

Fig. 3. Eccrine secretory tubule; note the coarse basophilic granules in the terminal cytoplasm of dark cells. From the scalp of a woman 38 years old. Ca. 1350×.

Fig. 4. Eccrine secretory tubule which shows the spurious two layered distribution of dark and clear cells. One of the nuclei is in metaphase. From the axilla of a man 29 years old. Ca. 1350×.

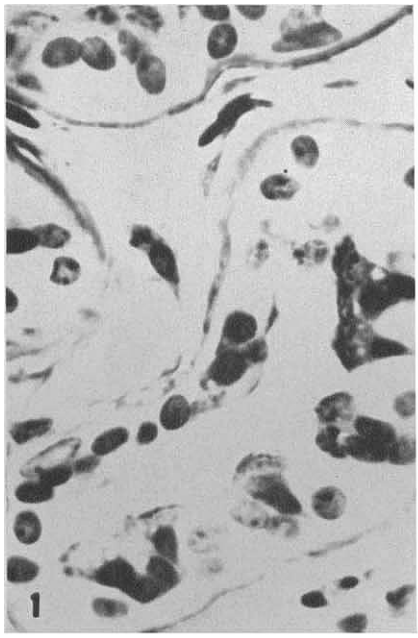

PLATE 1

thelium blends imperceptibly with the surrounding epidermis. The secretory coil as well as the whole duct is surrounded by areolar tissue rich in capillaries, venules and arterioles, and polymorphic mast cells.

The dark and clear secretory cells have vacuoles of different sizes in their cytoplasm (fig. 2). In occasional specimens nearly all of the secretory cells are riddled with vacuoles, showing the "clear reticular cytoplasm" described by Holyoke and Lobitz (9). The dark cells show only one or two small vacuoles above the nucleus, often touching the nuclear membrane. The clear cells show both inter- and intracellular canaliculi (10, 2, 11). The main branch of the intracellular canaliculi often curves around and below the nucleus, as observed also by Holmgren (10), and receives barely visible tributaries from the surrounding cytoplasm. Intercellular canaliculi receive lateral intracellular tributaries from surrounding cells. Contrary to the assertion of Ito and Iwashige (2) that cytoplasmic processes from "superficial" cells pinch off in the lumen in the fashion of "apocrine" cells, the free border of the cells is smooth, often showing a delicate cuticular border (fig. 2).

After staining with toluidin blue buffered to pH 5.0, the small, secretory cells are strongly basophilic but the large clear cells remain practically unstained (figures 1 to 4). The dark cells are full of delicate basophilic granules which become coarser at the terminal portion (fig. 3). The coarse granules show a metachromatic tinge (6). The larger cells, while apparently clear, contain barely visible basophilic granules gathered mostly around the nucleus. In sections digested with ribonuclease, all of the basophilic granules described are abolished.

In the transition zone of the duct, the cuboidal basal cells are diffusely basophilic. The superficial cells have below the cuticle and around the nucleus discrete basophilic granules with a tinge of metachromasia. Further up in the duct, where the surface cells have attained a well-developed cuticle, these cells no longer show basophilic granules. The basal cells remain strongly basophilic throughout the length of the duct. All of the basophilic elements described are removed by ribonuclease.

When stained with Giemsa stain the dark cells show at their apices, a mixture of basophilic and acidophilic granules. In the clear cell the cytoplasm contains numerous, extremely small, eosinophilic and basophilic granules.

Mitochondria in eccrine cells are usually coarse and sparse (fig. 9); they are in the form of short, stout rods, but filaments are occasionally found in the distal

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PLATE 2

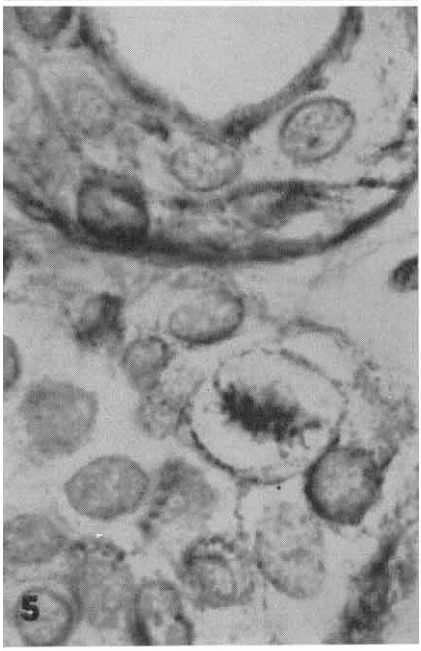
EXPLANATION OF FIGURES

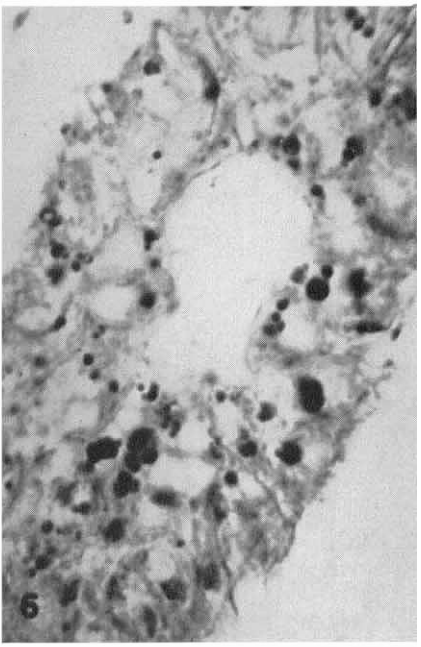
FIG. 5. Mitotic figure in transition zone of duct; lumen not in view. Stained with toluidin blue. From the axilla of a woman 29 years old. Ca. 1350×.

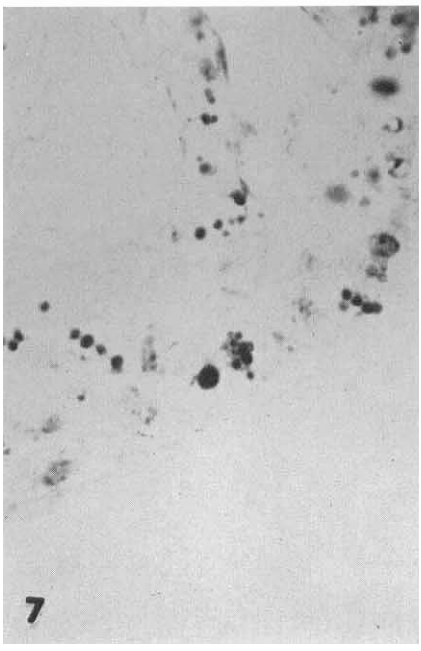
FIG. 6. Frozen section colored with Sudan black. From the axilla of a woman 32 years old. Ca. 675×.

FIG. 7. Paraffin section colored with Sudan black. From the axilla of a woman 32 years old. Ca. 675×.

FIG. 8. Frozen section treated with the acid hematein test for phospholipids. From the axilla of a woman 32 years old. Ca. 675×.







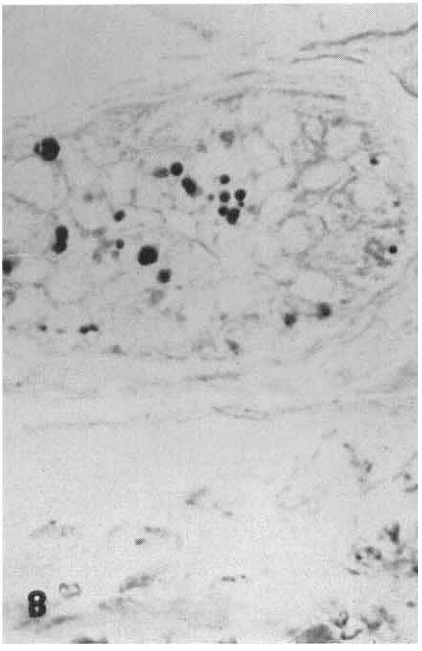
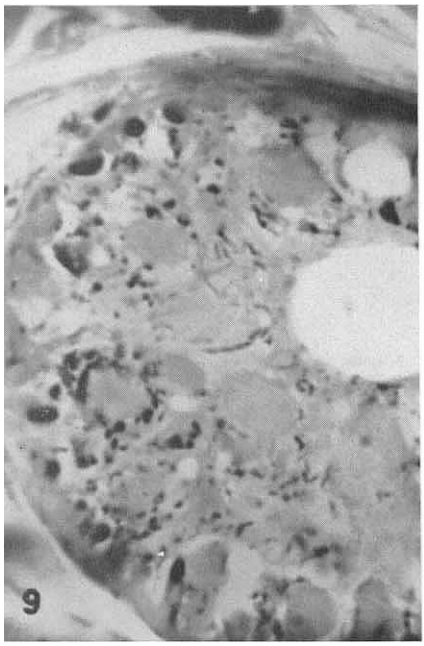
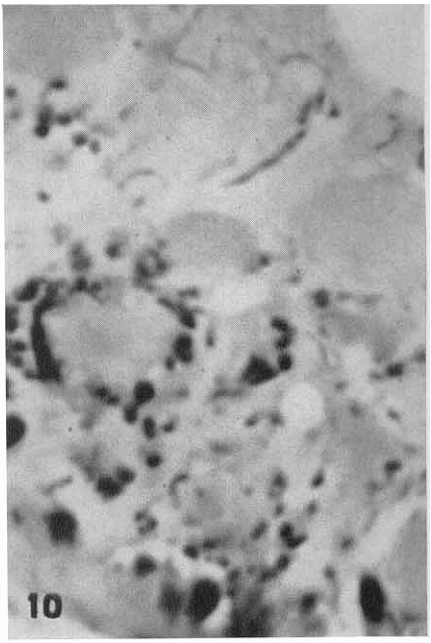
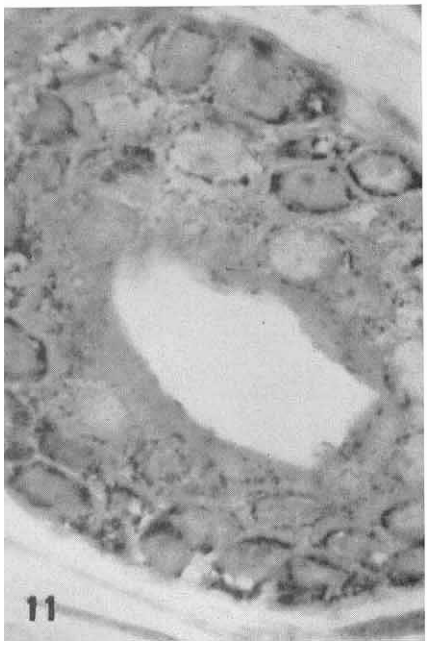


PLATE 2







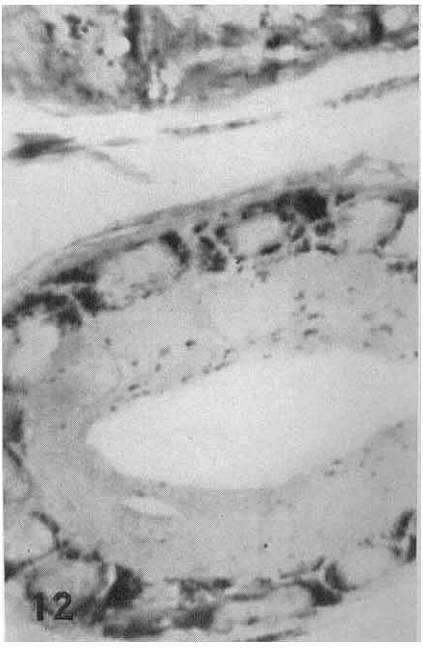


PLATE 3

cytoplasm (figures 9 and 10). In the clear cells rod-like mitochondria are scattered fairly evenly throughout the cytoplasm and bear no preferential relationship to the walls of the canaliculi. In the dark cells mitochondria are generally more numerous and filamentous. They are more prominent in the terminal cytoplasm where they are scattered among the cytoplasmic granules. The cells of the ducts abound in small, closely packed mitochondria (figures 11 and 12). In the cells of the basal layer they are numerous throughout the extent of the duct, whereas in the superficial cells they are usually clustered around the nucleus. In the transition zone, the superficial cells have a poorly developed cuticle and mitochondria are scattered in the supranuclear cytoplasm (fig. 11). Where the cuticle is well developed, the surface cells have hardly any mitochondria (fig. 12).

Glycogen in eccrine sweat glands has been described by several investigators (8, 12, 13, 11). Both dark and clear cells contain glycogen, but there is more of it in the clear cells. Some cells, scattered at random in the tubule are nearly free of glycogen. When inter- and intracellular canaliculi are seen distinctly, they are outlined by the glycogen granules dammed against their walls (11). In cells which do not contain glycogen, canaliculi are rarely found. The dark cells contain at their distal ends, coarse Schiff-reactive granules which are not digested by saliva (cf. Montagna et al.). The coarse, pigmented granules scattered in the cytoplasm of both types of cells are not reactive to the periodic acid-Schiff test. The cells of the duct are rich in glycogen (11, 12, 14). In the transitional zone the cells in both layers of the duct contain glycogen; in the middle portion glycogen is found only in the basal cells, and it is present again in all of the cells in the upper portion.

In their study of lipids, Bunting et al. (8) find a diffuse sudanophilia in the cytoplasm and discrete lipid granules which contain, in addition, an acetone-soluble, fluorescent pigment. These observations are correct, except that not much of the pigment is extracted with acetone, and some remains even in paraffin sections. The secretory cells contain variable numbers of lipid droplets (fig. 6), only some of which are soluble in organic solvents, whereas most of them resist extraction and are sudanophilic even in paraffin section (fig. 7). They are com-

PLATE 3

Explanation of Figures

The figures in this plate are from 3 μ sections of tissues fixed in Regaud's fluid and post-chromed 7 days at 37°C. The sections were stained with aniline acid fuchsin-methyl green

Fig. 9. Mitochondria in the cells of a secretory tubule. Ca. 2000X.

Fig. 10. Enlarged detail of fig. 9. Observe that the short rod-like mitochondria tend to cluster near the base of the cells and around the nucleus. In the terminal cytoplasm mitochondria tend to be filamentous. Ca. 4000×.

Fig. 11. Mitochondria in the transitional region of the duct are numerous around the nuclei, particularly in the cells of the basal layer. The cells of the surface layer have a poorly defined cuticle and contain numerous, delicate mitochondria. Ca. 2000×.

Fig. 12. Section of a duct, the superficial cells of which have a well developed cuticle. Mitochondria, numerous in the cells of the basal layer, are very scant in those of the superficial layer. Ca. 2000×.

posed largely of phospholipid (fig. 8), since they are reactive to Baker's (7) acid hematein test.

Cell death and replacement are not pronounced in eccrine glands. Mitosis in normal glands is neither common (8) nor rare (9). We have encountered at least a dozen mitotic figures in the specimens we have studied (figures 4 and 5).

DISCUSSION

The secretory coil of eccrine sweat glands is lined by two types of cells: small granular ones with nuclei usually located toward the lumen, and large clear ones with basally located nuclei (fig. 4). It is erroneous, however, to interpret these as "surface" and "basal" cells (2), since all of them, small and large, reach the basement membrane and have a free surface. When stained with basic dyes the small cells are intensely basophilic, but the large ones are not, and they are designated in this paper as dark and clear cells.

The dark cells contain numerous, often filamentous mitochondria; in the clear cells mitochondria are usually sparser and granular. Although both the dark and clear cells contain glycogen, it is more abundant in the clear cells. Only the apices of the dark cells contain Schiff-reactive, saliva-resistant granules. Vacuoles are larger and more numerous in the clear cells. Inter- and intracellular canaliculi are found principally in the clear cells. These facts suggest either that these cells are in different states of activity or that each type of cell secretes something different.

Some of the secretory cells contain coarse lipid granules (fig. 6) which are composed largely of phospholipid (fig. 8). Only some of these granules are dissolved by lipid solvents, most of them retaining their sudanophilia even after infiltration in paraffin (fig. 7); even some of the fluorescent pigment in them resists extraction by lipid solvents. The configuration and distribution of these lipid elements suggest that they are not a part of the Golgi complex or lipochondria. They appear, however, to be an inherent part of the cell since they are not eliminated in secretion (13).

Although glycogen is present in both clear and dark cells, it is usually more abundant in dark cells where it is often piled up against the walls of inter- and intracellular canaliculi (11). Since glycogen has been shown to disappear after prolonged intense sweating (13), it may be the source of energy used by the cells in secretion.

The excretory duct of eccrine glands is usually considered an indifferent passageway for sweat, yet its morphology seems to indicate an active function. Several authors (14, 12, 11) have shown that the cells of the ducts are rich in glycogen. There is an appreciable amount of ribonucleic acid in the cells of the duct, particularly in the surface cells where discrete granules of ribonucleic acid and traces of an acid polysaccharide are orderly arranged at the base around the nucleus. Both the basal and superficial layers of cells contain numerous mitochondria. These data strengthen the view of Lobitz and Mason (3) who suggest that "the coil of the sweat gland might act as a selective secretor and that the duct or the coil of the sweat gland, or both, may reabsorb water from the sweat."

SUMMARY

1. Two moderately distinct types of cells line the secretory tubules of eccrine glands; on the basis of their affinity for basic dyes they are designated as dark and clear cells. The smaller, dark cells contain numerous granules which are chiefly composed of ribonucleic acid; in the terminal cytoplasm these granules are very coarse. The larger, clear cells possess only sparse, very fine granules. Vacuoles and inter- and intracellular canaliculi are conspicuous only in the clear cells. Both dark and clear cells contain abundant glycogen and numerous mitochondria.

2. The secretory cells have in their cytoplasm lipid granules, most of them pigmented, which are reactive to Baker's test for phospholipids. Both lipid and pigment resist extraction in lipid solvents and are sudanophilic even after embedding in paraffin.

3. The cells of the lower portion of the excretory duct abound in ribonucleic acid, glycogen and mitochondria. These facts strongly suggest that duct may have an active secretory or reabsorbing function.

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